## REMARKS

Claims 1-3, and 9 have been withdrawn without prejudice or disclaimer of their later prosecution in one or more applications. Claims 4-8, 10, and 11 have been amended, as set forth above. Specifically, claim 4 has been amended to incorporate certain elements from non-elected claims. Claim 4 has also been amended to recite "wherein the polypeptide binds to antibody CR-50." Support for claim 4 can be found in original claims 4 and throughout the specification, for example, at page 12, lines 13-19.

Claim 5 has been amended to recite "and not containing a nucleotide sequence encoding an F-spondin domain or a repeat domain, and wherein the polypeptide encoded by the polynucleotide is capable of binding a CR-50 antibody." Claim 5 has also been amended to correct certain informalities in order to more clearly describe the claimed invention. Support for claim 5 can be found in original claim 5 and throughout the specification, for example, at page 4, lines 19-21.

Claims 8, 10, and 11 have been amended to correct claim dependencies.

Thus, the claims are fully supported by the application as originally filed and the amendment adds no new matter. With entry of this Amendment, claims 4-8 and 10-11 are pending for examination.

Specification

The Examiner objected to informalities in the Brief Description of the Drawings. *See Action* at page 2. Those informalities have been corrected, as set forth above.

Claim Objections

The Examiner objected to claims 4, 6-8, 10, and 11 for "depending in part from a withdrawn, non-elected invention." *Action* at page 2. The Examiner also objected to claims 10

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and 11 as allegedly reciting an improper Markush Group. *Id.* Solely to expedite prosecution and without acquiescing to the Examiner's contentions, Applicants have amended the claims to incorporate certain elements from non-elected claims and to correct claim dependencies. Also without acquiescing to the Examiner's contentions, claims 10 and 11 have been amended to correct the allegedly improper Markush Groups in those claims. Thus, the Examiner's objections are moot.

Rejections Under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 4-8, 10, and 11 under 35 U.S.C. § 112, first paragraph alleging that, "the specification, while being enabling for the polynucleotides of SEQ ID NO:1, does not reasonably provide enablement for polynucleotides of alternative sequence claimed including deletion, insertion, substitution and degenerate sequences." *Action* at page 3. Applicants respectfully traverse this rejection.

According to the Examiner, the "skilled artisan also recognizes that immunological responses depend upon the structural characteristics (conformation) of the particular protein (amino acid sequence) targeted and that variations in sequence may effect such structure and immunological recognition." *Action* at page 4. The Examiner then noted that certain claims recite "deletion, insertion, and substitution mutations as well as degenerate sequences." *Action* at page 5. The Examiner complained that "there is no disclosure of those residues which may be replaced, modified or deleted without abrogating the disclosed immunological reactivity." *Id.*

Applicants traverse. It is not necessary that the specification enable one skilled in the art to predict precisely which changes in a polypeptide will not affect activity, because modifying a polypeptide and testing it for activity does not involve undue experimentation. First, there can be no doubt that it was within the routine skill in the art at the time the application was filed to

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make polypeptides that are encoded by the nucleotide sequence of SEQ ID NO: 2. Second, it was within the routine skill in the art at the time the application was filed to make polypeptides that are encoded by a nucleotide sequence of SEQ ID NO. 2, comprising one or more additions, deletions, or substitutions. Finally it was within the routine skill in the art at the time the application was filed to determine if such polypeptides or polypeptide fragments bind to a CR-50 antibody as disclosed in the specification, for example at page 11, lines 15-21. Such determination can be accomplished by any of a variety of well-known techniques, including, but not limited to, Western blots. Moreover, such techniques have been routine in the art since well before the filing date of the instant application. *See, e.g.,* Ream and Field, *Molecular Biology Techniques: An Intensive Laboratory Course*, "D. Western Blot (Immunoblot) Detection of Proteins," Academic Press, p. 91-94 (1999) (copy enclosed). Furthermore, specific guidance for such techniques is provided in the specification, for example, at page 14.

Thus, the production of polypeptides or polypeptide fragments and the determination of their ability to bind CR-50 does not constitute undue experimentation. Instead, that process is simpler and less burdensome than the process of making and screening monoclonal antibodies, which the Federal Circuit found not to be undue experimentation in *In re Wands*, 858 F.3d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir.1988). In that case, the court held that the test for undue experimentation "is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine...." *Id.* at 737.

The *Wands* court further noted that "[t]he nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics." *Id.* at 740. As demonstrated above, protein biology (i.e., making and testing polypeptides and polypeptide fragments) also involves routine screening in order to find the

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polypeptides and polypeptide fragments with the desired activity. In fact, protein biology is arguably more predictable than screening hybridomas because each successive round of experiments may be directed according to the results of the previous experiments. For instance, at the time of filing, one skilled in the art would make a series of polypeptides and polypeptide fragments and determine whether each was capable of being bound by CR-50. The skilled artisan would then take only the polypeptides and polypeptide fragments that were capable of such binding and further alter them in a subsequent round of experiments. Moreover, one skilled in the art could deliberately make conservative alterations, which are well known in the art, and thus would have a reasonable expectation that the polypeptides and polypeptide fragments would retain binding ability. *See e.g.,* U.S. Pat. No. 5,739,277, to Presta et al., col. 14, line 4, through col. 15, line 64 (issued April 14, 1998) (copy enclosed).

This is in complete contrast to screening a random array of hybridomas, in which one skilled in the art has no directive input. Yet the Federal Circuit has found such screening of hybridomas not to involve undue experimentation. Accordingly, the making and screening polypeptides and polypeptide fragments for the ability to be bound by CR-50 antibody cannot involve undue experimentation.

The Examiner further alleged that claims 10 and 11 are not enabled because "the specification fails to teach such suitable compositions for stimulating the assembly of Reelin protein molecules or for providing a pharmaceutical for diagnosis or treatment of diseases resulting from abnormally positioned neurons." *Action* at page 5. Applicants respectfully traverse this rejection.

Claim 10 recites a "composition for stimulating the assembly of Reelin protein molecules." The specification provides adequate guidance to the skilled artisan to screen for

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such activity. For example, the specification discusses the use of Western blot analysis following both denaturing and non-denaturing gels. *See, e.g., Specification* at page 9, lines 24-page 10, line 6. Claim 11 recites a "pharmaceutical composition for diagnosis and/or treatment of diseases resulting from abnormally positioned neurons." The specification similarly provides adequate guidance to enable one of skill in the art to practice this claim, for example, at page 13, lines 8-18 (discussing certain diseases and providing exemplary methods). Finally, the Examiner alleged that "the model system is pertinent only to the Reelin phenotype which does not approximate all recognized abnormally positioned neurons but only those recognized as aberrantly positioned in Reelin animals." *Action* at page 5. Applicants respectfully traverse. The specification provides more than adequate guidance regarding certain diseases that may be treated and methods of treatment. For example, the specification states that:

> diseases resulting from abnormally positioned neurons, such as lissencephaly, polymicrogyria, and ectopic gray matter, may be treated by integrating a polynucleotide encoding the polypeptide of the present invention into an expression vector, introducing the vector into cells, and then transplanting the cells into the patient's brain.

*Specification* at page 13, lines 11-15. Accordingly, claims 10 and 11 are fully enabled by the specification as filed.

Finally, the Examiner based enablement rejections on an alleged lack of structure. *Action* at page 6. Specifically, the Examiner contended that the specification "provides essentially no guidance as to which of the essentially infinite possible choices is likely to be successful," and that "structure is critical to antibody binding." *Action* at page 6. Solely to expedite prosecution and without acquiescing to the Examiner's contention, Applicants have amended claims 4,-8, 10, and 11. The claims, as amended, all recite polynucleotides that encode polypeptides that are derived from a disclosed SEQ ID NO., or from a mouse Reelin protein. Further, the

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polypeptides must be capable of binding with a CR-50 antibody. Thus, the claims do recite structure and function. There is a finite number of polypeptides that combine such structure and such activity. For the reasons discussed, it is not overly burdensome to screen such proteins to determine whether they posses the necessary function. Thus, claims 4-8, 10, and 11 are fully enabled.

Applicants respectfully request reconsideration and withdrawal of the enablement rejection under 35 U.S.C. § 112, first paragraph.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 4-8, 10, and 11 were rejected under 35 U.S.C. for allegedly "failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention." *Action* at page 7. Specifically, the Examiner rejected claims 4-8, 10, and 11 as indefinite for depending from "withdrawn base claims." The dependency of those claims has been corrected.

The Examiner rejected claim 5 as indefinite for referring to "a degenerate of a polynucleic acid." *Action* at page 7. That claim has been amended to more accurately recite, "a degenerate of a polypeptide." Thus, the Examiner's rejections under 35 U.S.C. § 112, second paragraph are moot.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the enablement rejection under 35 U.S.C. § 112, second paragraph.

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1300 I Street, NW
Washington, DC 20005
202.408.4000
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Rejections Under 35 U.S.C. § 102

The Examiner rejected claims 4-8, 10, and 11, under 35 U.S.C. § 102(e) as allegedly being anticipated by Curran et al., US 6,323,177. *Action* at page 8. Applicants respectfully traverse this rejection.

For a reference to anticipate it must disclose every element of the claim. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986). Further, negative limitations are permitted. *See Manual of Patent Examining Procedure*, § 2173.05(i), Eighth Edition, (August 2001).

Claims 4 and 5 recite the negative limitation "not containing an F-spondin domain or a repeat site." Claims 6, 10 and 11 each recite a "polynucleotide according to claim 4 or 5." Claims 7 and 8 ultimately depend from claim 6. Thus, each of claims 4-8, 10, and 11 ultimately recites the negative limitation "not containing an F-spondin domain or a repeat site." Curran does not teach that negative limitation. In a passage cited by the Examiner, Curran discusses truncated Reelin peptides that "may be N-terminal, C-terminal, or they may contain internal fragments comprising some of the Reelin repeats." Col. 20, lines 4-6. That passage does not teach a Reelin polypeptide "not containing an F-spondin domain or a repeat site." To the contrary, it makes no mention of the F-spondin domain and specifically states that such peptides may include some of the Reelin repeats. Indeed, nowhere does Curran discuss the negative limitation of claims 4-8, 10, and 11. Accordingly, for at least that reason, Curran cannot anticipate claims 4-8, 10, and 11.

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1300 I Street, NW
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202.408.4000
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In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: April 14, 2003

By: _____
Clifford E. Ford
Reg. No. 52,903

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
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# MOLECULAR BIOLOGY TECHNIQUES

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**Walt Ream and Katharine G. Field**

Department of Microbiology
Oregon State University
Corvallis, Oregon

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# Contents

## Notes



# D. WESTERN BLOT (IMMUNOBLOT) DETECTION OF PROTEINS

## Introduction

The Western blot procedure is used to detect a specific protein among a mixture of proteins, or to show that a particular purified (or partially purified) protein is recognized by a specific antiserum. After SDS-PAGE, proteins are transferred by electrophoresis (also called electroblotting) to a nitrocellulose, nylon, or PVDF (polyvinylidene difluoride) membrane. A blocking agent binds nonspecific sites on the membrane. Primary antibodies raised against an antigen (the protein of interest) then bind specifically to protein antigens fixed to the membrane. The primary antibody is bound by a secondary antibody, which may be conjugated to a number of different "reporter" enzymes or molecules such as alkaline phosphatase, peroxidase, or biotin. In our experiment, the secondary antibody is conjugated to alkaline phosphatase; alkaline phosphatase activity is detected by conversion of a colorless substrate, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), into a blue indigo derivative.

Because the strength of the Western blot signal (color reaction) depends on both the affinity of the antibody for the antigen and the concentration of antigen present, the Western blot technique is **not** considered a quantitative measure of antigen concentration. An enzyme-linked immunosorbent assay (ELISA) **can** be used as a quantitative measure of antigen concentration. ELISAs are usually based on the same types of color reactions as immunoblots.

Two different types of antibody preparations are used for immunoblotting: polyclonal and monoclonal antibodies. Polyclonal antibodies are prepared by direct immunization of an animal with the antigen, and consist of the full repertoire of the animal's circulating antibodies. Po-

lyclonal antisera may contain antibodies highly specific for your antigen and others that recognize spurious antigens. Monoclonal antibodies are prepared by repeated direct immunizations of an animal followed by dissection of the animal's spleen (source of antibody-producing cells) and fusion of those spleen cells with a tumor cell line. The resulting monoclonal cell line expresses antibodies to a single antigenic epitope.

## Technical Tips

Nitrocellulose membranes are fragile and sensitive to fingerprints. **Pick up the membrane (in a corner) with clean forceps only.**

For experiments that use nylon or nitrocellulose membranes (Westerns, Southerns, Northerns), contaminants on the membrane cause significant background. Follow the times and temperatures for membrane washes in each of these procedures; skimping results in dirty blots.

## Protocol

1. After SDS-PAGE, place the unstained gel in 50 mL standard transfer buffer and rock for 10 minutes.
2. Cut the nitrocellulose filter to the size of the gel, and cut 2 pieces of Whatman 3 MM paper to the size (15 × 20 cm) of the Scotchbrite pads supplied with the electroblot apparatus (Mini-Genie Immunoblotters from Idea Scientific). Soak the pads, nitrocellulose, and Whatman 3 MM paper in transfer buffer. Do not touch the gel or any membranes or filters with ungloved hands.
3. Lay down 1 pad and cover with a sheet of Whatman 3 MM. Place the gel on the 3 MM paper and lay the nitrocellulose membrane over the gel. Lay a piece of Whatman 3 MM paper over the membrane. Use a pipet to roll out air bubbles between the gel and the membrane, and finally cover the entire sandwich with the other Scotchbrite pad.
4. Place the sandwich in the apparatus; it is extremely important that the sandwich is tight. Use additional Scotchbrite pads if necessary. Fill the chamber with transfer buffer, and apply 500 mA of current for approximately 30 minutes (this depends on the size of the protein you wish to transfer).
5. Wash the nitrocellulose membrane with rocking for 5 minutes in 15 mL distilled water (in a small plastic container).
6. Soak the membrane in 15 mL TBS for 10 minutes with rocking.
7. Soak membrane for 1 hour in 15 mL blocking solution (TBS + Tween 20 + gelatin), with rocking.
8. Pour off blocking solution. Wash the membrane in 15 mL TBS for 5 minutes with rocking.
9. Add 15 mL TBS. Add 15 μL of serum containing primary antibody (polyclonal antiserum raised in rabbit against glutathione-*S*-transferase antibody, 1 mg/mL); final concentration is 1 μg/mL in TBS. Mix thoroughly. Incubate, with rocking, at room temperature for 1 hour. This incubation can continue overnight.
10. Pour off buffer. Wash 3 times with 15 mL of TTBS for 5 minutes each with rocking.
11. Pour off buffer and add 15 mL TBS to dish. Now add 15 μL of secondary antibody (antiserum raised in goats against rabbit antibodies; the secondary antibody is conjugated to alkaline phosphatase). Incubate for 30 minutes at room temperature with rocking.

12. Pour off buffer, then wash 3 times in 15 mL TTBS for 5 minutes each.
13. Pour off buffer and replace with 15 mL TBS. Leave in TBS until ready to add substrate.
14. Pour off last wash, then add 10 mL of substrate solution. Incubate until color reaction is sufficient.
15. Rinse blot in distilled water. Dry on Whatman paper.

## Solutions for Western Blot

Transfer buffer: 25 mM Tris (3 g/L Tris base)
192 mM glycine (14.4 g/L)
20% methanol (200 mL/L)
0.1% SDS (1 mL/L)

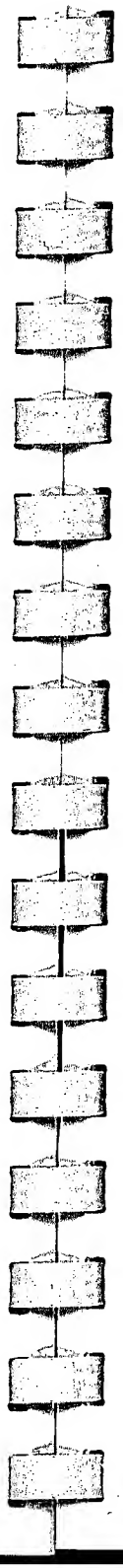
TBS (Tris-buffered saline): 20 mM Tris, pH 7.5
150 mM NaCl

TTBS: TBS + 0.1% Tween 20

Blocking solution: TBS
0.1% Tween 20
1% gelatin

Primary antibody: Upstate Biotechnologies anti-Gst; purified polyclonal IgG antibody raised in rabbits against Gst expressed by pGEX2.

ICN Western Blot Staining Kit: this kit supplies secondary antibody (goat polyclonal antiserum made against rabbit antigens), and color staining reagents (nitro blue tetrazolium and BCIP).





## Notes